

**WHAT IS CLAIMED IS:**

1. A method for regulating the expression of a transgene of interest *in vivo* comprising:

5 - simultaneously introducing into a target nonhuman animal tissue or cell a first nucleic acid comprising the sequence of a transgene of interest encoding a transcript of interest, and a second nucleic acid comprising the sequence of an inhibitory transgene encoding an inhibitory transcript specific for the transcript of interest, wherein each of the sequences are under the control of a transcriptional promoter, and the activity of the inhibitory transcript is optionally regulated with at least one external agent, and the activity of the transcript of interest is optionally regulated with at least one external agent, and

10 - coexpressing said nucleic acids in the target tissue or cell to constitutively inhibit the activity of the transcript of interest with the inhibitory transcript.

2. The method according to Claim 1, further comprising administering to the target nonhuman animal tissue or cell at least one external agent which causes the activity of said inhibitory transcript to be inhibited, for the purpose of restoring the activity of the transcript of interest.

20 3. The method according to Claim 2, further comprising administering to the target nonhuman animal tissue or cells at least one external agent which causes the activity of said transcript of interest to be increased, for the purpose of restoring the activity of the transcript of interest.

25 4. The method according to Claim 1, further comprising administering to the target nonhuman animal tissue or cells at least one external

agent which causes the activity of said transcript of interest to be increased, for the purpose of restoring the activity of the transcript of interest.

5           5.       The method according to Claim 1, wherein the inhibitory transcript is under the control of a repressible promoter, and at least one external agent causes the inhibition of said promoter.

          6.       The method according to Claim 5, wherein the promoter comprises at least one repeat of the tetracycline response operator, and at least one external agent is an external repressor agent chosen from tetracycline and tetracycline analogues.

10           7.       The method according to Claim 1, wherein the nucleic acid encoding the inhibitory transcript also comprises an activatable autocatalytic aptamer sequence, and the method comprises at least one external agent which is a specific ligand of the aptamer and which is an activator of the aptamer.

15           8.       The method according to Claim 1, wherein the nucleic acid encoding the inhibitory transcript also comprises a sequence which can be recognized by a ribozyme with ligand-dependent activity, and the external agent is a ligand which is an activator of the catalytic activity of the ribozyme.

20           9.       The method according to Claim 1, wherein the sequence encoding the transcript of interest is placed under the control of an inducible promoter, and the method comprises at least one external agent which causes the activation of said promoter.

          10.      The method according to Claim 9, wherein the inducible promoter comprises a PPAR $\alpha$  response element, and wherein at least one external agent is a PPAR $\alpha$  ligand.

11. The method according to Claim 10, wherein the inducible promoter comprises a PPAR $\alpha$  response element, and at least one external agent is a PPAR $\alpha$  ligand chosen from fibrates and fibrate analogues.

12. The method according to Claim 9, wherein the inducible promoter comprises a PPAR $\gamma$  response element, and the method comprises at least one external agent which is a PPAR $\gamma$  ligand.

13. The method according to Claim 12, wherein the inducible promoter comprises a PPAR $\gamma$  response element, and at least one external agent is a PPAR $\gamma$  ligand chosen from fatty acids and thiazolidinediones.

14. The method according to Claim 1, wherein the inhibitory transcript is in the form of an antisense RNA which is complementary to at least one coding portion of the mRNA of the transgene of interest, and the inhibitory transcript is capable of forming with the at least one coding portion of the mRNA of the transgene of interest a Watson and Crick-type linkage.

15. The method according to Claim 1, wherein the inhibitory transcript is in the form of an antisense RNA which is complementary to at least one noncoding portion of the mRNA of the transgene of interest, and the inhibitory transcript is capable of forming with the at least one noncoding portion of the mRNA of the transgene of interest a Watson and Crick-type linkage.

16. The method according to Claim 15, wherein the inhibitory transcript is in the form of an antisense RNA which is complementary to at least one noncoding portion at the 5' end of the mRNA of the transgene of interest, and the inhibitory transcript is capable of forming with the at least one noncoding portion at the 5' end of the mRNA of the transgene of interest a Watson and Crick-type linkage.

17. the method according to Claim 15, wherein the antisense RNA is at least 10 ribonucleotides long.

18. The method according to Claim 1, wherein the inhibitory transcript is in the form of an RNA capable of forming a triple helix with a portion of  
5 the nucleic acid comprising the sequence of the transgene of interest.

19. The method according to Claim 1, wherein the inhibitory transcript is in the form of a ribozyme.

20. The method according to Claim 1, wherein the transgene of interest encodes a protein of therapeutic interest.

10 21. The method according to Claim 1, wherein the transgene of interest corrects a genetic abnormality or of deficiency.

22. The method according to Claim 1, wherein the transgene of interest encodes an activator which is involved in the expression of another gene.

15 23. The method according to Claim 1, wherein the transgene of interest produces a transcript of interest having therapeutic activity.

24. The method according to Claim 1, wherein the nucleic acids are carried by a single vector or different vectors.

25. The method according to Claim 24, wherein the nucleic acids are carried by the same strand of the same vector.

20 26. The method according to Claim 24, wherein the vector is a plasmid, a cosmid, an artificial chromosome or a nonencapsidated DNA.

27. The method according to Claim 24, wherein the vector is a recombinant virus.

25 28. The method according to Claim 27, wherein the recombinant virus is an adenovirus, a retrovirus, a herpesvirus, an adeno-associated virus, a phage or a derivative of these.

29. The method according to Claim 24, wherein the vector is a bacterium or parasite.

30. The method according to Claim 1, wherein the nucleic acids are introduced into the target tissue or cell using a physical or mechanical method.

5 31. The method according to Claim 30, wherein the nucleic acids are introduced into the target tissue or cell using a physical or mechanical method chosen from injection, ballistic technique, electroporation, sonoporation, exposure to an electrical field, exposure to microwaves, exposure to heat or pressure, or by a combination of these techniques.

10 32. The method according to Claim 30, wherein the nucleic acids are introduced into the target tissue or cell by injection, electrotransfer, or a combination thereof.

15 33. The method according to Claim 1, wherein the nucleic acids are introduced into the target tissue or cell in a form which is complexed with at least one chemical agent or biochemical agent.

34. The method according to Claim 33, wherein the at least one chemical agent or biochemical agent is a cationic protein chosen from histones and protamines.

20 35. The method according to Claim 33, wherein the at least one chemical agent or biochemical agent is a polymer chosen from DEAE-dextran, polyamidoamines, polylysines, polyethyleneimines, polyvinylpyrrolidones or polyvinyl alcohols.

36. The method according to Claim 33, wherein the nucleic acids are incorporated into lipids in crude form or in the form of liposomes.

25 37. The method according to Claim 33, wherein the nucleic acids are incorporated into nanoparticles.

38. The method according to Claim 1, wherein the target tissue or cell is of nonhuman animal or human origin.

39. The method according to Claim 38, wherein said target tissue or cell of nonhuman animal or human origin is a muscle cell or a skeletal muscle  
5 tissue.

40. The method according to Claim 1, wherein the target tissue or cell is of plant origin.

41. The method according to Claim 30, wherein the nucleic acids are injected systemically.

10 42. The method according to Claim 41, wherein the nucleic acids are injected intra-arterially or injected intravenously.

43. The method according to Claim 42, wherein the nucleic acids are present in a composition comprising pharmaceutically acceptable excipients for the intra-arterial or intravenous methods of administration.

15 44. The method according to Claim 30, wherein the nucleic acids are administered parenterally, topically, cutaneously, vaginally, intranasally, subcutaneously, intra-ocularly, or a combination thereof.

45. The method according to Claim 44, wherein the nucleic acids are present in a composition comprising pharmaceutically acceptable excipients for  
20 the parenteral, topical, cutaneous, vaginal, intranasal, subcutaneous or intra-ocular methods of administration.

46. A composition comprising a first nucleic acid comprising a sequence of a transgene of interest encoding a transcript of interest, and comprising a second nucleic acid comprising a sequence of an inhibitory transgene encoding an  
25 inhibitory transcript specific for said transcript of interest, wherein the sequences are each under the control of a transcriptional promoter, and wherein the activity of the

inhibitory transcript is optionally regulated with at least one external agent and the activity of the transcript of interest is optionally regulated with at least one external agent.

47. The composition according to Claim 46, wherein the inhibitory  
5 transcript is in the form of an antisense RNA which is complementary to at least one coding portion of the mRNA of the transgene of interest, and wherein the antisense RNA is capable of forming with the at least one coding portion of the mRNA of the transgene of interest a Watson and Crick-type linkage.

48. The composition according to Claim 46, wherein the inhibitory  
10 transcript is in the form of an antisense RNA which is complementary to at least one noncoding portion of the mRNA of the transgene of interest, and wherein the antisense RNA is capable of forming with the at least one noncoding portion of the mRNA of the transgene of interest a Watson and Crick-type linkage.

49. The composition according to Claim 48, wherein the inhibitory  
15 transcript is in the form of an antisense RNA which is complementary to at least one 5' noncoding portion of the mRNA of the transgene of interest, and wherein the antisense RNA is capable of forming with the at least one 5' noncoding portion of the mRNA of the transgene of interest a Watson and Crick-type linkage.

50. The composition according to Claim 46, wherein the antisense  
20 RNA is at least 10 ribonucleotides long.

51. The composition according to Claim 46, wherein the inhibitory transcript is in the form of an RNA capable of forming a triple helix with a portion of the nucleic acid comprising the sequence of the transgene of interest.

52. The composition according to Claim 46, wherein the inhibitory  
25 transcript is in the form of a ribozyme.

53. The composition according to Claim 46, wherein the sequence encoding the inhibitory transcript is under the control of a repressible promoter, and wherein at least one external agent causes the inhibition of said promoter.

54. The composition according to Claim 53, wherein the promoter  
5 comprises at least one repeat of a tetracycline response operator, and at least one external agent is at least one external repressor agent chosen from tetracycline and tetracycline analogues.

55. The composition according to Claim 46, wherein the nucleic acid encoding the inhibitory transcript also comprises an activatable autocatalytic  
10 aptamer sequence, and wherein the composition further comprises at least one external agent which is a specific ligand of the aptamer and is an activator of the aptamer.

56. The composition according to Claim 46, wherein the nucleic acid encoding the inhibitory transcript also comprises a sequence which can be  
15 recognized by a ribozyme with ligand-dependent activity, and wherein the composition further comprises at least one external agent which is a ligand of the ribozyme and is an activator of the catalytic activity of the ribozyme.

57. The composition according to Claim 46, wherein the sequence of the transgene of interest encoding the transcript of interest is placed under the  
20 control of an inducible promoter, and at least one external agent causes the activation of said promoter.

58. The composition according to Claim 57, wherein the inducible promoter comprises a PPAR $\alpha$  response element, and at least one external agent is a PPAR $\alpha$  ligand.



59. The composition according to Claim 58, wherein the inducible promoter comprises a PPAR $\alpha$  response element, and at least one external agent is a PPAR $\alpha$  ligand chosen from fibrates and fibrate analogues.

60. The composition according to Claim 57, wherein the inducible  
5 promoter comprises a PPAR $\gamma$  response element, and at least one external agent is a PPAR $\gamma$  ligand.

61. The composition according to Claim 60, wherein the inducible promoter comprises a PPAR $\gamma$  response element, and at least one external agent is a PPAR $\gamma$  ligand chosen from fatty acids and thiazolidinediones.

10 62. The composition according to Claim 46, wherein the transgene of interest encodes a protein of therapeutic interest.

63. The composition according to Claim 46, wherein the transgene of interest corrects a genetic abnormality or deficiency.

15 64. The composition according to Claim 46, wherein the transgene of interest encodes an activator which is involved in the expression of another gene.

65. The composition according to Claim 46, wherein the transgene of interest produces a transcript of interest having therapeutic activity.

66. The composition according to Claim 46, wherein the nucleic acids are carried by a single vector or different vectors.

20 67. The composition according to Claim 66, wherein the nucleic acids are carried on the same strand of the same vector.

68. The composition according to Claim 66, wherein the vector is a plasmid, a cosmid, an artificial chromosome or a nonencapsidated DNA.

25 69. The composition according to Claim 66, wherein the vector is a recombinant virus.

70. The composition according to Claim 69, wherein the vector is a recombinant virus chosen from adenoviruses, retroviruses, herpesviruses, adeno-associated viruses, phages, and derivatives thereof.

5 71. The composition according to Claim 66, wherein the vector is a bacterium or a parasite.

72. The composition according to Claim 46, wherein the composition is in a form wherein the nucleic acids can be administered *in vivo* to the target tissue or cell using a physical or mechanical technique.

10 73. The composition according to Claim 72, wherein the composition is in a form wherein the nucleic acids can be administered *in vivo* to the target tissue or cell using a physical or mechanical method chosen from injection, ballistic technique, electroporation, sonoporation, exposure to an electrical field, exposure to microwaves, exposure to heat or pressure, or by a combination of these techniques.

15 74. The composition according to Claim 73, wherein the composition is in a form wherein the nucleic acids can be administered by injection, electrotransfer, or a combination thereof.

75. A method for regulating a transgene of interest in a target cell or tissue, comprising the composition as defined in Claim 46.

20 77. The method according to Claim 75, wherein the target cell or tissue is of nonhuman animal origin or human origin.

78. The method according to Claim 75, wherein the target cell or tissue is a muscle cell or muscle tissue.

25 79. The method according to Claim 75, wherein the target cell or tissue is of plant origin.

80. A pharmaceutical composition comprising the composition according to Claim 46 and a suitable pharmaceutical excipient.

81. The pharmaceutical composition according to Claim 80 in a form that can be injected into muscle, injected systemically, injected intra-arterially  
5 or injected intravenously.

82. The pharmaceutical composition according to Claim 80 in a form that can be administered topically, cutaneously, orally, vaginally, intranasally, subcutaneously or parenterally.

83. The pharmaceutical composition according to Claim 80,  
10 wherein the excipient is an excipient that can be injected into muscle, injected systemically, injected intra-arterially or injected intravenously, or the excipient can be administered topically, cutaneously, orally, vaginally, intranasally, subcutaneously, or parenterally.

84. A method for manufacturing a medicinal product intended for  
15 correcting a genetic abnormality or deficiency, comprising the composition according to Claim 46.

85. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating mitochondrial genetic diseases.

20 86. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating myopathies.

87. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating ischemias and stenosis.

25 88. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating lysosomal diseases.

89. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating hormonal disorders.

90. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating hemophilia.

5 91. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating inflammatory diseases or disorders.

10 92. The method according to Claim 91, wherein the medicinal product is intended for treating the inflammatory disease chosen from rheumatoid arthritis.

93. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating  $\beta$ -thalassemia.

94. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended to induce apoptosis.

15 95. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for anticancer treatment.

96. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating neurodegenerative diseases.

20 97. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating cardiovascular diseases or disorders.

25 98. The method according to Claim 97, wherein the medicinal product is intended for treating the cardiovascular disease or disorder chosen from hypertension.

99. A method of using the composition according to Claim 46, for manufacturing a medicinal product intended for treating hyperlipidemias.

100. The method according to Claim 99, wherein the medicinal product is intended for treating the hyperlipidemia chosen from obesity

5 101. A method of using the composition according to Claim 46, for manufacturing vaccines.

102. A transgenic animal, wherein the animal carries a first nucleic acid comprising the sequence of a transgene of interest encoding a transcript of interest, and a second nucleic acid comprising the sequence of an inhibitory  
10 transgene encoding an inhibitory transcript specific for the transcript of interest, wherein each of the sequences are under the control of a transcriptional promoter, and the activity of the inhibitory transcript is optionally regulated with at least one external agent, and the activity of the transcript of interest is optionally regulated with at least one external agent.

15 103. The transgenic animal according to Claim 102, wherein the inhibitory transcript is in the form of a genetic antisense RNA, of an RNA capable of forming a triple helix or of a ribozyme.

20 104. The transgenic animal according to Claim 102, wherein the inhibitory transcript is regulated negatively via a repressible promoter, and at least one external agent causes the inhibition of said promoter.

105. The transgenic animal according to Claim 102, wherein the inhibitory transcript also comprises an activatable autocatalytic aptamer sequence, and at least one external agent is a specific ligand of the aptamer sequence which is an activator of the aptamer sequence.

25 106. The transgenic animal according to Claim 102, wherein the inhibitory transcript also comprises a sequence which can be recognized by a

ribozyme with ligand-dependent activity, and at least one external agent is a ligand of the ribozyme which is an activator of the catalytic activity of the ribozyme.

107. The transgenic animal according to Claim 102, wherein the transcript of interest is under the control of an inducible promoter, and at least one  
5 external agent causes the activation of said promoter.

108. A transgenic plant, wherein the plant carries a first nucleic acid comprising the sequence of a transgene of interest encoding a transcript of interest, and a second nucleic acid comprising the sequence of an inhibitory transgene encoding an inhibitory transcript specific for said transcript of interest, wherein each  
10 of the sequences are under the control of separate transcriptional promoters, and the activity of the inhibitory transcript is optionally regulated with at least one external agent, and the activity of the transcript of interest is optionally regulated with at least one external agent.

109. The transgenic plant according to Claim 108, wherein the  
15 inhibitory transcript is in the form of a genetic antisense RNA, of an RNA capable of forming a triple helix or of a ribozyme.

110. The transgenic plant according to Claim 108, wherein the inhibitory transcript is regulated negatively via a repressible promoter, and at least one external agent causes the inhibition of said promoter.

20 111. The transgenic plant according to Claim 108, wherein the inhibitory transcript also comprises an activatable autocatalytic aptamer sequence, and at least one external agent is a specific ligand of this aptamer sequence.

112. The transgenic plant according to Claim 108, wherein the inhibitory transcript also comprises a sequence which can be recognized by a  
25 ribozyme with ligand-dependent activity, and at least one external agent is a ligand of the ribozyme which is an activator of the catalytic activity of the ribozyme.

113. The transgenic plant according to Claim 108, wherein the transcript of interest is under the control of an inducible promoter and at least one external agent causes the activation of said promoter.

113. The transgenic plant according to Claim 108, wherein the transcript of interest is under the control of an inducible promoter and at least one external agent causes the activation of said promoter.